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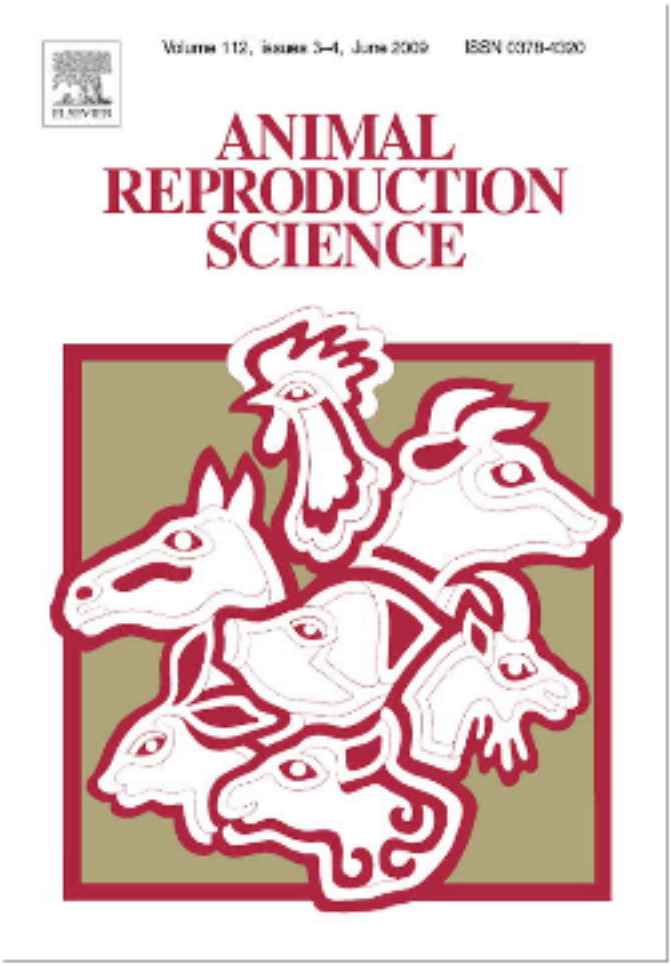

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Longitudinal monitoring of plasma and fecal androgens in the Tasmanian devil (*Sarcophilus harrisii*) and the spotted-tailed quoll (*Dasyurus maculatus*)

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Abstract

Improved knowledge of the breeding biology of carnivorous marsupials is warranted given their heightened conservation status. Past studies have focused on smaller dasyurids and little is known of male reproductive physiology in the larger species. This study aimed to characterize the pattern of androgen concentrations in male devils and spotted-tailed quolls and to evaluate fecal steroid measurement as a practical, alternative technique for monitoring reproductive activity. Blood and fecal samples were collected from captive adult devils ($n=6$) and adult quolls ($n=8$). Plasma and fecal androgen concentrations were significantly positively correlated. In both species there was a significant effect of season on androgen concentrations; and the annual increase preceded female estrus activity. For devils, fecal androgens were elevated during the austral summer: peak concentrations were observed in January–February, and copulation occurred from late February–late May. In quolls, fecal androgen concentrations were highest during austral autumn/winter: the annual increase began in April and copulation occurred from mid-May to early October. The lengthy period of elevated plasma and fecal androgens and protracted annual period of mating activity implies a period of extended spermatogenesis in both species.
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Keywords: Marsupial; Dasyurid; Testosterone; Hormones; Fecal steroids

1. Introduction

The Tasmanian devil (*Sarcophilus harrisii*) and the spotted-tailed quoll (*Dasyurus maculatus*) are the largest surviving carnivorous marsupials (max 7–12 kg: Strahan, 2005) and both species are

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native to Australia. These closely related dasyurids now co-exist only on the island of Tasmania, and both are on state and national Threatened species lists. Spotted-tailed quolls are naturally rare across most of their range, and are primarily endangered by habitat loss (Jones et al., 2003). Devils became extinct on the Australian mainland ~400–5000 years ago (Jones et al., 2003) but remained widespread and common in Tasmania until very recently; the species is currently facing serious risk of extinction from a rapidly spreading, and contagious fatal disease (Hawkins et al., 2006; Pearce and Swift, 2006). For both species, there is an obvious necessity to maintain self-sustaining captive insurance populations; however, this is particularly challenging in dasyurids because of their naturally short reproductive life span (1–3 years) and low breeding output (Jones et al., 2003). Zoos have had limited breeding success with either species. The current lack of information on their reproductive biology is a major factor in these low success rates (Williams, 1990; Carnio, 1993; Jackson, 2003).

Despite a strong research focus on the breeding biology of the smaller carnivorous marsupials (Tyndale-Biscoe and Renfree, 1987), until recently only basic life history variables were available for the devil and the spotted-tailed quoll (Fleay, 1952; Guiler, 1970; Settle, 1978; Manserg, 1984; Belcher, 2003). Following mating and a short gestation (~3 weeks) a single, relatively small litter (4 or 6 young, respectively) are weaned in spring (Fleay, 1935, 1940; Green, 1967; Guiler, 1970; Belcher, 2003). Dasyurids typically have a well-regulated annual breeding period (McAllan, 2003), yet in the devil and spotted-tailed quoll there is some intriguing evidence that out-of-phase breeding can occur in the wild (Guiler, 1970; Green and Scarborough, 1990; Körtner, 2006). We have recently characterized the estrous cycle in female devils and spotted-tailed quolls (Hesterman et al., 2008a,b), but there is still no published information on the endocrinology of male reproduction for either species.

Dasyurids exhibit a range of reproductive strategies, relating in part to the timing and frequency of female estrous cycles and also the timing and extent of male effort (Lee et al., 1982; Lee and Cockburn, 1985). Documenting male androgen rhythms is a necessary step towards understanding breeding synchrony between the sexes. The annual pattern of plasma testosterone concentrations has been reported for a range of male marsupials (reviewed in Tyndale-Biscoe and Renfree, 1987) including several other quoll species (Bryant, 1986; Schmitt et al., 1989), phascogales (*Phascogale*) (Bradley, 1987; Millis et al., 1999), and the well-studied dunnart (*Sminthopsis*) and marsupial mouse (*Antechinus*) (McDonald et al., 1981; Wilson and Bourne, 1984). Such studies have traditionally measured concentrations of total androgens or testosterone in plasma. An obvious advantage of developing a non-invasive method for measurement of androgens is to circumvent potential problems caused by the stress of repeated handling, which has been reported to depress peripheral testosterone concentrations in several marsupial species (Lincoln, 1978; Curlewis and Stone, 1984). Despite the popularity of non-invasive sex steroid measurement in eutherian mammals (reviewed in Lasley and Kirkpatrick, 1991; Schwarzenberger et al., 1996) and application of these techniques to monitoring reproduction in female marsupials (Stead-Richardson et al., 2001; Paris et al., 2002; Oates et al., 2004; Hesterman et al., 2008a,b), the only published study on male marsupials (Hamilton et al., 2000) is an investigation of fecal androgens in southern hairy-nosed wombats (*Lasiorhinus latifrons*).

The primary aims of the present study were: (1) to characterize the annual pattern of androgen concentrations in male devils and spotted-tailed quolls and (2) to evaluate fecal steroid monitoring as an alternative, non-invasive technique for monitoring testicular rhythms in these dasyurid species.

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2. Materials and methods

2.1. Study animals and husbandry

Samples were collected from six adult male devils (2–8 years old) and eight adult male spotted-tailed quolls (1–4 years old) housed at Trowunna Wildlife Park (TWP, Mole Creek, Tasmania) between May 2000 and December 2001. All devils and one quoll were from an already established captive population. Seven male quolls were trapped from the wild between August 2000 and April 2001, and relocated to the park under permit. They were captured in wire-cage carnivore traps, aged, sexed, inspected for health, and individually marked by ear tattoo prior to being brought into captivity.

Devils and quolls were fed a natural meat-based diet consisting mainly of kangaroo or wallaby and occasionally possum or rabbit. Spotted-tailed quolls received additional items including a prepared mix of grated carrot, apple, pumpkin seeds, egg and insectivore mix (Wombaroo Food Company, Mt Barker, SA). Additional enrichment food items provided less often included commercially available brands of dog or cat biscuits. Water was available *ad libitum*.

Study animals were housed either in outdoor enclosures or pens with outside access, except for two quolls, # 02 and # 07—which were kept indoors under a natural lighting regime for 6–9 weeks, respectively, for purpose of display. Animals kept outside were housed on natural substrate; those indoors were maintained on a wooden floor spread with eucalyptus mulch. All had access to climbing structures, native plants and other natural materials. Dens or nest boxes were available for shelter, and the number of retreats provided was greater than or equal to the number of animals per enclosure.

Grouping of the study animals varied during the year according to accepted husbandry management for the species. Male devils were housed individually during the breeding season (January–June) but were maintained in mixed sex groups at other times of the year. Spotted-tailed quolls were routinely housed individually because of this species' more solitary nature (Belcher and Darrant, 2004). Three of the male devils (3/6) and four of the male quolls (4/8) were paired with individual females for breeding purposes (Table 1) as required by husbandry at the captive facility. Pairing duration was dependent on female receptivity. Following mating and/or lack of interest or increased aggression toward males, females were removed to separate enclosures. Estrus periods of female devils and quolls were determined through endocrine monitoring, as part of concurrent studies; copulations were confirmed by behavioural observation (observer presence/video monitoring within dens), or detection of sperm in a vaginal smear (Hesterman et al., 2008a,b).

2.2. Plasma collection

Samples were taken between 15:00 and 17:00 h to reduce potential complications of diurnal variation in plasma testosterone concentrations. Animals were captured by hand or with the use of a large net, and restrained unanaesthetised in a sack during sample collection. Blood was collected within 5 min of capture. A peripheral ear vein was pricked with a disposable Stat-Let© lancet and 50–100 μL blood was collected via a heparinised capillary tube. Samples were kept at 4 °C until centrifuged later that day, and the plasma separated and stored frozen (−20 °C) until radioimmunoassay. Plasma collection focussed on the period encompassing the main breeding season for each species. Blood was collected from five of the devils (2–4 years old) at intervals of ~7–10 days during late January–July 2001, but not from the single, aged individual (8 years old)

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Table 1
Details of pairing and breeding activity in male Tasmanian devils (*Sarcophilus harrisii*) and spotted-tailed quolls (*Dasyurus maculatus*).

Species	Studbook/ID #	Paired for breeding	Pairing period[a]
T. Devil	126	No	
	203	Yes	22–25 May
	272	Yes	21 February–22 May
	295	No	
	329	No	
	666	Yes	23 March
S.T. Quoll	1	Yes	18–21 June; 16–18 July
	2	Yes	18/19 July year 1; 9/10 August year 2
	3	Yes	12 August
	4	No	
	5	Yes	18/19 June; 1–3 October
	6	No	
	7	No	
	8	No	

[a] Range of dates during which males were housed with estrus females.

(#126) (Table 1). Quolls were bled at ~3–4-week intervals during April–September 2001; due to the nervous disposition of this species (HH, personal observation), blood sampling was limited to four males only.

2.3. Fecal collection

Fecal samples were collected weekly from all study animals. To identify individual samples when animals were housed together, small colored plastic beads were mixed into a mincemeat ball and fed to study animals the previous day. Entire fecal samples were collected during morning servicing (07:30–09:00 h) or opportunistically when freshly voided throughout the day. When several scats were available from the same individual, the most visibly fresh sample was selected. Samples were placed in zip-lock plastic bags and stored at −20 °C for later processing. Frozen samples were freeze-dried (Dynavac FD16, Dynavac High Vacuum Pty Ltd., Victoria, Australia) and sieved through 1 mm^2 plastic mesh to remove fur, bones and other fibrous or undigested matter; the screened feces were refrozen until analysis.

2.4. Sample analyses

2.4.1. Plasma

Plasma androgen was analysed using an already-established radioimmunoassay procedure (Pankhurst and Conroy, 1987). In brief, duplicates of 50 μL of plasma were extracted in 1 mL ethyl acetate (AnalaR grade Merck Pty, VIC, Australia) by vortexing and the sealed tubes left to incubate at room temperature for 90 min to maximise extraction. Samples were centrifuged for 5 min at 3000 rpm, and the solvent was recovered and evaporated under air. Evaporated plasma extracts were reconstituted in 200 μL phosphate buffer (0.05 M containing 0.1% gelatin) and vortexed prior to radioimmunoassay (RIA). A serial dilution (range 3–400 pg/mL in buffer) of testosterone standards was prepared (T-1500 Sigma–Aldrich Pty, Ltd., Missouri, USA) and replicate 200 μL of each dilution used to create a standard curve. 200 μL of [^{3}H] testosterone ([1,2,6,7]